International Journal of Agricultural
Science and Research (IJASR)
ISSN(P): 2250-0057; ISSN(E): 2321-0087
Vol. 6, Issue 4, Aug 2016, 161-164


# ROLE OF STOMATAL FREQUENCY IN PLANT INNATE IMMUNITY AGAINST BACTERIAL BLIGHT DISEASE OF POMEGRANATE

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***ABSTRACT***

***Decline in area and production of pomegranate is evinced since 2002 due to severe outbreak of bacterial blight diseases caused by Xanthomonas axonopodis pv. punicae. Stomata have been claimed as a major portal of entry for bacterial blight pathogen; hence an attempt has been made to find out the correlation between stomatal frequency and their reaction to bacterial blight. In the present investigation, stomatal density and their influence on pathogen reaction was studied using ten genotypes. The study revealed that stomatal number was significantly less in resistant varieties as compared to susceptible ones. Further, significant and positive correlation was also observed between stomatal frequency and bacterial blight incidence.***

***KEYWORDS: Pomegranate, Bacterial Blight, Xanthomonas Axonopodis Pv. Punicae, Stomatal Frequncy***

**Received:** Jun 23, 2016; **Accepted:** Jul 21, 2016; **Published:** Jul 23, 2016; **Paper Id.:** IJASRAUG201622

Original Article

## INTRODUCTION

Pomegranate cultivation gained its momentum worldwide due to its renowned potentiality in imports and exports. India ranked first with its area of 1, 31,000 hectares and production and productivity of 13, 45,000 metric tons and 10.3 tons/ha, respectively (Anon., 2014). Nevertheless, decline in pomegranate cultivation is averred from the year 2002 due to occurrence of epidemic disease caused by *Xanthomonas axonopodis* pv. *punicae*. The pathogen entry into pomegranate tissues is adjudged by the symptoms that appear on leaves, fruits and even on mature branches. Small, irregular, water soaked, lesions with yellow hallow appears on leaves, which form a bigger brown patches on disease progression and later drop off. Fruits on infection develop circular brown to black lesions with 'L'/'Y' or star shaped cracks and on stems, the disease starts as brown to black spot around the nodes and in advance stages, girdling and cracking of nodes lead to break down of branches (Singh *et al.*, 2015). The bacterial blight disease caused by *Xanthomonas axonopodis* pv. *punicae* is primarily air-borne, the pathogen ingress and invades the host through stomata or any pores and cannot breach intact leaf surfaces. It is also reported that bacteria lack the ability to directly penetrate the plant epidermis; they rely entirely on natural openings or accidental wounds to enter internal tissues of hosts. *Xanthomonas axonopodis* pv. *punicae* as residents of the leaf surface probably can be splashed spread to other leaves, infecting through stomata. A possible relationship between stomatal frequency and the resistance to pathogen entry has also been established during plant-pathogen interactions (Rich, 1983). However, the study on stomatal density in spread of bacterial diseases in plants is limited. Thus, this study focussed on stomatal frequency and its foremost role in bacterial blight disease of pomegranate.

## MATERIALS AND METHODS

The study was carried out at the Division of fruit crops, Indian Institute of Horticultural Research (IIHR), Bengaluru. A total of ten genotypes of pomegranate were selected, screened for bacterial blight disease, calculated stomatal frequency and correlated with bacterial blight disease to understand the mechanism of disease spread.

- **Screening of Bacterial Blight Diseases**: Screening was done following detached leaf method as described by Verniere *et al.* (1998) and Mondal and Kumar (2011). A total of ten genotypes were screened and details of the plant material used in the study are presented in Table 1. Twenty five leaves per genotype were inoculated by pin-prick method and placed on moist filter paper in a sterile petri-plate. Three replications were maintained for each genotype in a randomized block design and incubated at 28 ±2°C. Observations on bacterial blight incidence (mm), onset of necrotic symptoms (days), number of leaves infected (%), disease scoring and reaction were recorded for 10 days. The scoring of disease was made following a scale presented by Gopalakrishnan et al. (2009) and Mondal and Kumar (2011).

- **Stomatal Frequency:** Stomatal count was determined by fevicol method as depicted by Divya *et al.* (2014). Fevicol was smeared on adaxial surface of leaf to form a thin film. After five minutes, fevicol layer was peeled off and mounted on clean slide and observed under microscope. Number of stomata present in lower surface was recorded at 10×40 magnifications and expressed in terms of number of stomata per 0.009 sq mm area of leaf surface.

- **Data Analysis**: Statistical analyses were performed using SPSS package (SPSS Inc. version 16.0) for all sets of data, means were compared using Duncan multiple comparison test at P = 0.01 and correlated between stomatal frequency and disease parameters.

## RESULTS AND DISCUSSIONS

Ten genotypes were screened for bacterial blight under *in-vitro* conditions. Screening in laboratory was done following pin prick method as it showed high pathogenic potential compared to other methods (Eknath *et al.*, 2015). Among all the genotypes screened, it was evinced that the genotype IC 318734, a wild collection from sub-Himalayan region with bacterial blight incidence of 0.26mm, onset of necrotic symptoms ($10^{th}$ day) and leaves infected (5%) imparted more resistance compared to others Table 2. The results were in consonant with the results published by Benagi and Kumar (2009) and Singh *et al* (2015), where they determined that cultivated types are more prone to bacterial blight as compared to wild-type accessions. This might be supported by stating that plants bearing fruits with higher acidity generally exhibit lower bacterial blight incidence. Singh *et al* (2015) also reported that 153-bp allele; prevalent in wild types is associated with less bacterial blight severity and high fruit acidity. Sporadic and infrequent differences in disease severity across the genotypes denoted the differential resistant responses exhibited by various genotypes (Prajongjai *et al.* 2014) and this might be attributed to a gene-to-gene relationship (Flor, 1956)

Photo-micrographic view revealed that the stomatal density was lower in resistant genotype IC 318734 (51.80) and higher in susceptible type Ruby (157.20), highlighting a constitutive system of defense mechanism Table 2. Bacterial plant pathogens enter host tissue through natural surface openings such as stomata, hydathodes, lenticels, wounds etc. Among natural openings, stomata dominate in number in the aerial part of the plant and therefore represent one of the most important routes for the entry of foliar bacterial pathogens (Melotto *et al.* 2008). Significant and positive correlation

observed between stomatal number and bacterial blight incidence, leaves infected while negative correlation with onset of necrotic symptoms Table 3. The fewer number of stomatal pores found in genotype IC 318734 would limit the entry of pathogen and confer resistance as evident from the Table 3. Similar observation was recorded with bacterial plant diseases by Shukla and Gangopadhyaya (1981) and Ramos and Volin (1987).

## CONCLUSIONS

The genotypes tested shown wide variability in stomatal frequency that contributed in exploring plant innate immunity. Genotypes with higher number of stomata succumbed to susceptibility of plant diseases and *vice-versa*. Among the 10 genotypes tested, IC 318734 with lesser frequency of stomata and lower disease incidence was found to bestow resistance for bacterial blight disease in pomegranate.

## ACKNOWLEDGMENTS

The authors are thankful to the Director, Indian Institute of Horticultural Research (IIHR), Bengaluru, for providing research facilities and funding the above research work. Special thanks are due to Dr. A. Rekha for her guidance in conducting stomatal studies.

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## APPENDICES

**Table 1: List of Studied Pomegranate Accession with IC/EC Number and Place of Collection**

| S. No. | Accession Name | IC/EC Number | Place of Collection | GPS (Global Positioning System) | |
|---|---|---|---|---|---|
| | | | | Latitude | Longitude |
| 1. | 318734 | IC318734 | HIMALAYAN REGION, INDIA | 28° 35’ 53.94^N | 83°55’ 51.82^E |
| 2. | Daru-18 | - | HIMALAYAN REGION, INDIA | 28° 35’ 53.94^N | 83°55’ 51.82^E |
| 3. | 99 | EC798793 | BALKAN, TURKMENISTAN | 39° 31’ 25.94^N | 54°20’ 9.60^E |
| 4. | IIHR-30 | - | IIHR, INDIA | 13° 7’ 55.36^N | 77°29’ 21.37^E |
| 5. | 108 | EC798801 | TURKMENISTAN | 38° 58’ 10.99^N | 59°33’ 22.60^E |
| 6. | Daru | - | IIHR, INDIA | 13° 7’ 55.36^N | 77°29’ 21.37^E |
| 7. | Ruby | - | IIHR, INDIA | 13° 7’ 55.36^N | 77°29’ 21.37^E |
| 8. | Bhagwa | - | IIHR, INDIA | 13° 7’ 55.36^N | 77°29’ 21.37^E |
| 9. | Muskat | - | IIHR, INDIA | 13° 7’ 55.36^N | 77°29’ 21.37^E |
| 10. | P-23 | - | IIHR, INDIA | 13° 7’ 55.36^N | 77°29’ 21.37^E |

**Table 2: Frequency of Stomata on Adaxial Surface of Leaf with Disease Incidences and Its Factors Responsible for Bacterial Blight Disease in Pomegranate**

| S. No. | Genotype | Disease Incidence (mm) | Onset of Necrotic Symptoms (Days) | Leaves Infected (%) | Disease Scoring | Stomatal Number |
|---|---|---|---|---|---|---|
| 1. | IC 318734 | 0.26 | 10 | 5 | 1 | 51.8 |
| 2. | Daru-18 | 0.32 | 10 | 18 | 1 | 55.4 |
| 3. | 99 | 0.72 | 10 | 6 | 1 | 55.6 |
| 4. | IIHR-30 | 0.5 | 9.6 | 15 | 1 | 71.6 |
| 5. | 108 | 0.44 | 9.6 | 15 | 1 | 85.2 |
| 6. | Daru | 3.06 | 7.6 | 30 | 4 | 85.8 |
| 7. | Ruby | 66.16 | 2 | 100 | 5 | 157.2 |
| 8. | Bhagwa | 42.36 | 6 | 100 | 5 | 138 |
| 9. | Muskat | 27.44 | 6 | 94 | 5 | 98.8 |
| 10. | P-23 | 7.92 | 6 | 85 | 5 | 96.2 |
| | **Sem±** | 0.69 | 0.42 | 6.87 | | 2.8 |
| | **CD** | 2.64 | 1.61 | 26.28 | | 10.71 |

**Table 3: Correlation of Stomatal Frequency and Bacterial Blight Disease Factors in Pomegranate**

| Particulars | Stomatal Number | Bacterial Blight Incidence | Onset of Necrotic Symptoms | Leaves Infected |
|---|---|---|---|---|
| **Stomatal Number** | 1.00 | 0.93 | -0.92 | 0.87 |
| **Bacterial Blight Incidence** | * | 1.00 | -0.91 | 0.83 |
| **Onset Of Necrotic Symptoms** | * | * | 1.00 | -0.90 |
| **Leaves Infected** | * | * | * | 1.00 |